The Use of Genetically Modified Animals in Carcinogenicity Bioassays

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INTRODUCTION

After almost 4 decades of conducting carcinogenicity bioassays in conventional rats and mice, the International Conference on Harmonization of testing requirements for registration of pharmaceuticals for human use has prompted a serious consideration of genetically modified mouse models for identifying carcinogen potential (3). These new models are expected to suffer from the same fundamental problem of all models—there is no ideal surrogate for humans in identifying human carcinogens. Nonetheless, considerable effort is being expended by several groups to evaluate the potential utility of genetically modified mice as carcinogenesis models (4). Up to now, the approach has been largely empirical, with the most promising results being derived from testing of genotoxic carcinogens. Critical studies are currently underway to test the utility of 3 popular models in identifying a spectrum of agents with less clear carcinogenic potency.

THREE CURRENTLY FASHIONABLE GENETICALLY ALTERED MODELS

The 3 genetically altered mouse models that are available commercially and for which testing results under standardized protocols have been published are the Tg.AC transgenic mouse and the *p53*$^{\pm}$ knock-out mouse (both available commercially in the United States) and the rasH2 transgenic mouse (available in Japan from the Central Institute for Experimental Animals). All 3 models are based upon genetic factors (viz, the Ha-*ras* gene and the *p53* tumor suppressor gene) relevant to many human and rodent tumors, and all can develop a robust tumor response during a 6-month treatment regimen with few or no tumors in concurrent vehicle or untreated controls. The initial fear that these models would be overly responsive has proven unfounded.

The Tg.AC mouse is an appealing model because it responds to both genotoxic and nongenotoxic carcinogens, but it may be the most controversial model because of its unclear mechanistic basis and because the preferred route of exposure is by skin painting, with skin papillomas as the reporter phenotype. Other routes of exposure, however, are being explored; this model may be systemically responsive, with development of tumors at certain sites other than painted skin. One troubling recent observation is an apparent sequence alteration in the transgene, leading to loss of responsiveness in some hemizygous Tg.AC mice. Homozygous animals appear to respond more uniformly, but only time will tell if they will also show instability of the transgene. Inclusion of a positive control group and saving tail snips for retrospective genomic analysis, should an unexpected result occur, are wise precautions for studies involving Tg.AC mice. Although the rasH2 and *p53*$^{\pm}$ mice are responsive to genotoxic carcinogens, more testing results are needed to assess adequately their responsiveness to nongenotoxic carcinogens.

Several articles on the use of these and other genetically altered mice have been published in a recent issue of *Toxicologic Pathology* (4). These articles reflect various national and international governmental and industrial efforts currently underway to evaluate and validate these alternative models.

SOME GENERAL RESERVATIONS

Although the potential for use of genetically altered mice in carcinogen identification is exciting, use of these models should be approached with some reservation. There is no a priori reason to expect that any new model will give all the correct answers all the time. Ultimately, we must define the mechanisms underlying the observed responses to use intelligently the information generated by the new models. In practice, underlying mechanisms may be difficult to define until we have some confidence that the new models are accurately predictive. Other concerns about the use of these models have been previously presented (1). The first few false-positive or false-negative results will likely precipitate a rash of mechanistic studies to offer explanations. Without such elucidation, we could witness the demise of any model for use in the hazard identification arena.

Another reservation concerns the fact that these models are inherently artificial, especially those that have had exogenous genetic material inserted into the genome. The

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impact that the exogenous genetic material exerts on the model performance may be related to the foreign genetic material itself or to some cryptic perturbation of the functioning of intrinsic key regulatory genes elsewhere in the genome. Without knowing the mechanism by which the model is working, overwhelming empirical evidence that the model does work will be required, even if we do not know exactly why it works. The database must become sufficient over time for investigators to be comfortable with an empirically based model. Furthermore, the exogenous genetic material may provoke instability in the genome, resulting in altered responsiveness of the model. We require assurances that when the model gives a negative result the agent has little or no carcinogenic potential, not that genetic instability has resulted in nonresponders.

Testing protocols utilizing genetically altered models have been continually expanding in magnitude and scope. What were originally touted by some as quick 6-month studies with minimal histopathology are evolving into investigations requiring complete histopathology, inclusion of positive controls, inclusion of nontransgenic littermates or wild-type controls, tests to insure the stability of the genotype, and the possible extension of the studies beyond 6 months and perhaps requiring more animals. Given the hefty purchase price of the animals plus the incrementally expanding protocols, the cost advantage over the conventional 2-year bioassay will be diminished, especially if studies involve multiple models. Despite the expanding protocols, use of genetically altered mice for hazard identification will likely remain faster and less expensive than conventional rodent 2-year bioassays. Also, some scientists who are critical of conventional 2-year mouse bioassays seem ready to accept the prospect of testing their chemicals in the new designer mice. Other scientists, however, maintain a healthy skepticism.

Realistically and inevitably, a tendency to compare the results in any new model with those for which a larger database exists will develop. Comparisons of new model results with those from conventional chronic rodent cancer bioassays will be generated, despite the fact that the conventional bioassay may not be a true gold standard. With all its imperfections, the chronic rodent cancer bioassay may be the best that we have for identifying carcinogenic potential of agents with unknown human carcinogenicity. For any new model to be successful and thrive, it must either be as good as the older model or be proven to identify more accurately human carcinogens. If there is a lack of concordance between any new model and the older models, we may be faced with costly and time-consuming mechanistic studies to offer explanations or with the prospect of abandoning the model for hazard identification, as occurred with the strain A mouse pulmonary tumor model.

Proposed Purpose of Model Usage

Of paramount importance in considering the use of any new model, be that model a transgenic, a knock-out, a knock-out : knock-in, or any other of the proposed alternative models, is the need to clearly articulate the proposed purpose of model useage. Although very specific research purposes can be defined for model useage, there are at least 3 general purposes: to identify carcinogenic potential, to learn about the biological effects of a chemical, and to learn about mechanisms of toxicity or carcinogenicity.

Identifying Carcinogenic Potential

A primary driving force behind the contemporary validation studies involving genetically altered mice is the desire for a quicker and less costly cancer bioassay model to replace or supplant the conventional mouse 2-year cancer bioassay. Initial use of these models has clearly shown that they respond to potent known carcinogens, a condition necessary for early consideration of any potential new cancer bioassay model. Although the ideal approach would be to discover a cancer bioassay model that accurately discriminated between agents carcinogenic to humans and those agents that are not carcinogenic, there are an insufficient number of known human carcinogens that lend themselves to animal testing to permit sufficient confidence in the models. By default, then, the proposed new genetically altered models are ultimately being validated against known genotoxic and nongenotoxic rodent carcinogens and appropriate noncarcinogens that have been adequately tested in conventional rodent models. Thus, despite the limitations of the conventional rat and mouse 2-year cancer bioassay, it tends to become a standard against which a proposed new model is compared. As can be expected, any new model will undoubtedly fail to yield results totally concordant with those of the conventional rodent bioassay. If a bioassay in a proposed new model is positive, it will be considered to have conditionally identified carcinogenic potential. If it is negative, the results will not likely be accepted without additional information about the agent under investigation and results in other test systems. Once a sufficient number of known carcinogenic and noncarcinogenic agents have been tested in a proposed new model, the model could gain acceptance as a viable model for identifying carcinogenic potential. The 30–50 studies that may be required to reach an appropriate comfort level with respect to the performance of the proposed new model will undoubtedly limit the number of potential models that will be used for the purpose of identifying carcinogenic potential.

Learning about the Biological Effects of a Chemical

Genetically altered mice may, indeed, be highly useful in studies of known carcinogenic agents. Study of sequential pathogenesis and the effects of potential preventive or therapeutic measures would represent a pragmatic use of a model that yields a carcinogenic response in 6 months.

Learning about the Mechanisms of Toxicity or Carcinogenicity

Specific genetic alterations allow for the study of toxicity and carcinogenicity as it is influenced by either overexpression or underexpression of specific genes. The use of genetically altered mice for this purpose is ideal and, in fact, is very often the reason a particular trans-

genic or knock-out mouse was developed in the first place. Once the effect of gene expression is known in the untreated transgenic or knock-out mouse, potential mechanisms can be deduced, and subsequent studies with known toxicants or carcinogens may permit elucidation of similar or dissimilar mechanisms of action.

CARCINOGEN HAZARD IDENTIFICATION APPLICATIONS UNDER CONSIDERATION

Testing Only in Genetically Altered Model(s)

At this stage in the development and use of genetically engineered mice, testing of an unknown agent for carcinogenic potential will only be useful for decisionmaking if the outcome is positive and if the mechanism of tumor induction is biologically relevant. A model that overresponds will not be useful for decisionmaking if there is a suspicion that the response is a false positive or if the mechanism is unique to that particular genetically altered mouse. It is unlikely that negative tests derived from studies only in genetically altered mice will achieve regulatory acceptance unless the agent is closely related to similar agents that have been extensively studied previously. Under these conditions, regulatory acceptance will likely be determined on a case-by-case basis.

Testing in Genetically Altered Models as a First-Tier Approach

Screening chemicals for carcinogenic potential prior to conducting more conventional carcinogenicity studies represents a possible use of genetically altered mice that could lead to modifications in subsequent study design that would permit generation of additional data for use in risk assessment. Initial screening tests in relevant transgenic or knock-out mice would allow for important decisions in drug development strategy. This first-tier approach would also be useful for testing of suspected carcinogens or analogs of known carcinogens.

Use of Genetically Altered Models as a Second Species in Testing Carcinogenic Potential

A primary consideration in using genetically engineered mouse models as a second species in carcinogen hazard identification is to utilize the models in lieu of conventional mouse 2-year carcinogenesis testing. This potential use is receiving significant consideration by the pharmaceutical industry and some regulatory authorities and may be an appropriate use of these animal models because the pharmaceutical under development generally has been well characterized in terms of pharmacologic, physiologic, metabolic, and nonneoplastic pathologic effects. However, many environmental agents, including industrial chemicals and pesticides, have not been well characterized. Hence, regulatory authorities dealing with these agents have not enthusiastically endorsed the use of genetically altered models as potential replacements for 2-year cancer bioassays in conventional mice.

Use of Genetically Altered Models as a Third Species in Testing Carcinogenic Potential

The use of genetically engineered mice following 2-year cancer bioassays in conventional rats and mice may be warranted to assess dose-response, to further examine a tissue-specific response, or to study several related chemical congeners. On a case-by-case basis, use of transgenic or knock-out mice to help clarify an equivocal response in conventional animal bioassays or a response that is problematic with respect to human relevance may be considered. This type of application has already been used twice by the National Toxicology Program (NTP) in response to requests from the US Food and Drug Administration. Regulatory decisions or label changes were made based upon the additional data provided by a study using genetically altered mice.

Use of Genetically Altered Models as a Modified Prechronic Study Preceeding Conventional 2-Year Rodent Bioassays

NTP scientists are considering a recent proposal made by Dr Raymond Tennant of the National Institute of Environmental Health Sciences. The proposal is that a 6-month study in transgenic or knock-out mice be used in place of the 90-day prechronic study typically performed to characterize toxicity and help set doses for a 2-year cancer bioassay. Such a study would better characterize toxicity because it would last 6 months instead of 3 months, and if the chemical had carcinogenic potential, that might also be evident prior to the start of 2-year conventional animal testing protocol. A decision could be made to proceed with the 2-year conventional rodent bioassays in light of the findings in the genetically engineered mice, to modify the 2-year bioassays based upon a preneoplastic or neoplastic response in the genetically modified model, to do a 2-year bioassay in only 1 species, or possibly to do no further testing when all information is taken into consideration. Data regarding strain sensitivity, metabolism, and toxicokinetics will be necessary in considering this proposed use of genetically modified mice.

INFLUENCE OF STRAIN BACKGROUND

What is already apparent from the initial validation studies of genetically altered mouse models is that the background strain significantly influences the observed response. Depending upon the transgene or the silenced gene, the strain effect may be dominant. For some of the background strains used for development of genetically modified mice (eg, FVB), there is little information on metabolism or spontaneous tumor incidences. Once the genome has been altered, strain characteristics may change. For example, *p53*$^{\pm}$ mice have a higher frequency of sarcomas than do the background C57BL/6 strain, and rasH2 transgenic mice develop splenic hemangiosarcomas whereas these neoplasms are rare in the $CB6F_1$ background hybrid. As these genetically modified mouse models are further utilized, there will be a need to define metabolic pathways, characterize spontaneous nonneoplastic and neoplastic lesions, assess dose-responsiveness of the new models, explore comparative toxicokinetics, and address issues such as why chemicals that induce tumors at certain sites in 2-year conventional mouse bioassays are missed by the new models.

Conclusions

The opportunities afforded by current efforts to develop alternative models such as genetically altered mice include the opportunity for improving how potential human carcinogens are identified and strengthening the science base for risk assessment. Certain fundamental questions remain. How is a new model developed? What constitutes development? Are the new models more reflective of human risk than conventional models? When and how should data from emerging models be utilized?

An ideal model is reliable, relevant, rapid, and economical and has international regulatory acceptance. Model validation is the "process by which the reliability and relevance of a test method are established for a specific purpose" (2). Because validation studies in genetically modified mice are ongoing, it is not known how ideal the currently popular genetically altered mouse models will be. In another year, a sufficient database will have been generated to permit an informed assessment of the strengths and weaknesses of these mice for purposes of hazard identification. Until more results are available, it is recommended that these models be used in a knowledge-driven manner for clearly defined purposes, and if positive, the results may be used for risk assessment. Although genetically engineered mouse models have already contributed greatly to our understanding of several important aspects of carcinogenesis, there is no escape from good scientific judgment in using these models for carcinogen hazard identification.

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